Isolation of primitive endoderm, mesoderm, vascular endothelial and trophoblast progenitors from human pluripotent stem cells.

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Public Summary:
Identification of developmental progenitors (intermediates between pluripotent stem cells and tissue cells) holds a promise for enabling purification of therapeutic preparations of tissue regenerating cells, and for molecular modeling diseases in culture conditions. The aim of this study is to define cell surface markers of human progenitors which can be used as molecular beacons for purifying the progenitors from heterogeneous preparations of differentiated human embryonic stem cells (hESCs). We screened over 400 markers using specific antibodies against BMP4-treated and retinoic acid-treated cultures that promote cell specification in cultured hESCs. We discovered and purified over 30 subpopulations followed by transcriptional analysis of developmental genes. Gene profiling indicated the identification of four distinct candidate progenitor groups. Subsets detected in self-renewing cultures expressed primitive endoderm genes indicating differentiation towards yolk sac fate. Progenitors emerging following BMP4 treatment exhibited gene signatures of mesoderm, trophoblast and vascular endothelium, suggesting that they have the capacity to give rise to cardiovascular cells, placenta and umbilical cord vasculature, respectively. Furthermore, we performed functional studies in vitro and in vivo confirmed to confirm these cell fate associations. Finally we showed that these progenitor populations and respective markers emerge from patient-derived induced pluripotent stem cells (iPS cells). Taken together, these findings pave the way for translational studies utilizing the progenitors as tools for purifying human tissue-regenerating progenitors, and for basic studies aiming to decipher how PSCs create the variety of cell lineages in the human embryo. These data also facilitate clinical translation by providing markers to deplete tumor-initiating cells from heterogeneous mixtures of differentiated cells that emerge from hES and iPS cells.

Scientific Abstract:
To identify early populations of committed progenitors derived from human embryonic stem cells (hESCs), we screened self-renewing, BMP4-treated and retinoic acid-treated cultures with >400 antibodies recognizing cell-surface antigens. Sorting of >30 subpopulations followed by transcriptional analysis of developmental genes identified four distinct candidate progenitor groups. Subsets detected in self-renewing cultures, including CXCR4(+) cells, expressed primitive endoderm genes. Expression of Cxcr4 in primitive endoderm was confirmed in visceral endoderm of mouse embryos. BMP4-induced progenitors exhibited gene signatures of mesoderm, trophoblast and vascular endothelium, suggesting correspondence to gastrulation-stage primitive streak, chorion and allantois precursors, respectively. Functional studies in vitro and in vivo confirmed that ROR2(+) cells produce mesoderm progeny, APA(+) cells generate syncytiotrophoblasts and CD87(+) cells give rise to vasculature. The same progenitor classes emerged during the differentiation of human induced pluripotent stem cells (hiPSCs). These markers and progenitors provide tools for purifying human tissue-regenerating progenitors and for studying the commitment of pluripotent stem cells to lineage progenitors.